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Swartz et al.

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(54) LOCATION ANALYSIS USING FIRE RETARDANT-PROTECTED NUCLEIC ACID-LABELED TAGS

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- (51) **Int. Cl. G01N 37/00** (2006.01) **C12Q 1/68** (2006.01)
- (58) Field of Classification Search
 NoneSee application file for complete search history.

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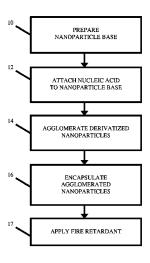
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(57) ABSTRACT

A nucleic acid tag comprising a nucleotide-support platform attached to a nucleic acid molecule, a fire retardant, and an encapsulant. Unique nucleic acid-containing tags containing a fire- or heat-protective element are seeded at one or more geographic locations. Using sequence analysis techniques, the person or object of interest is examined for the presence of one or more of the seeded nucleic acids. The geographic location associated with each detected nucleic acid is used to backtrack the item's path or extrapolate a probable point of origin.

8 Claims, 15 Drawing Sheets



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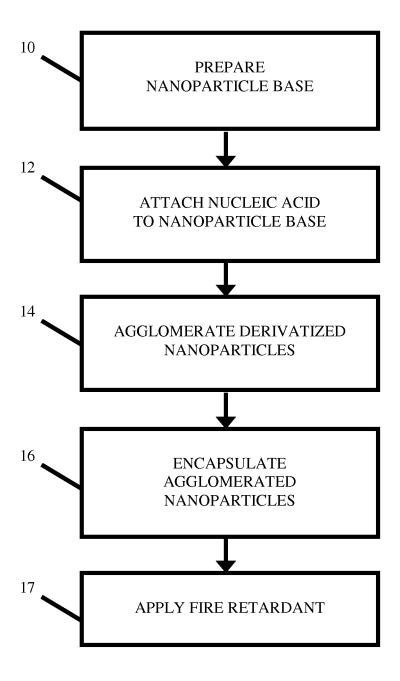


FIG. 1

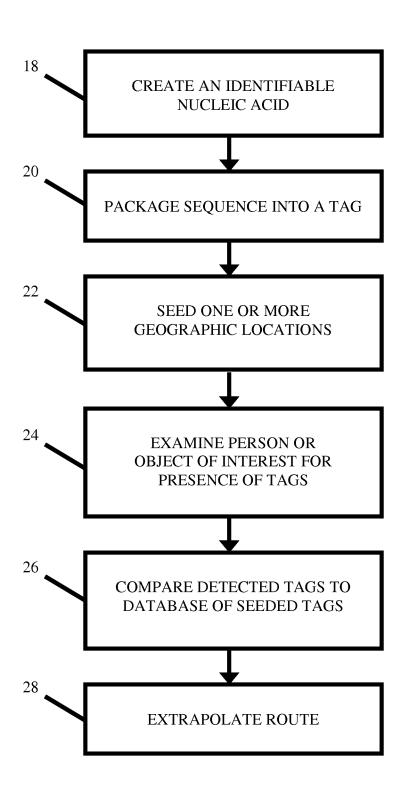


FIG. 2

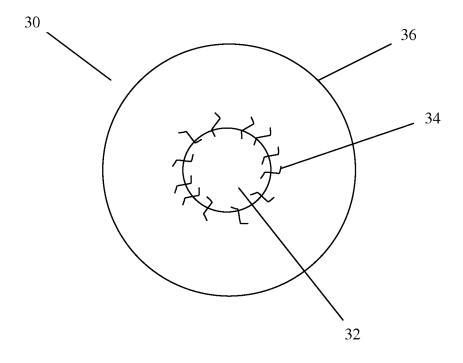


FIG. 3

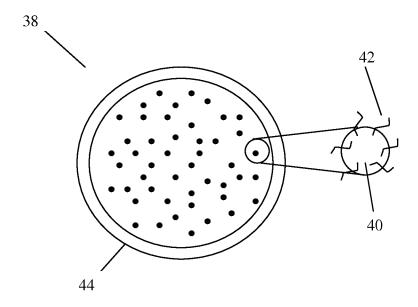


FIG. 4

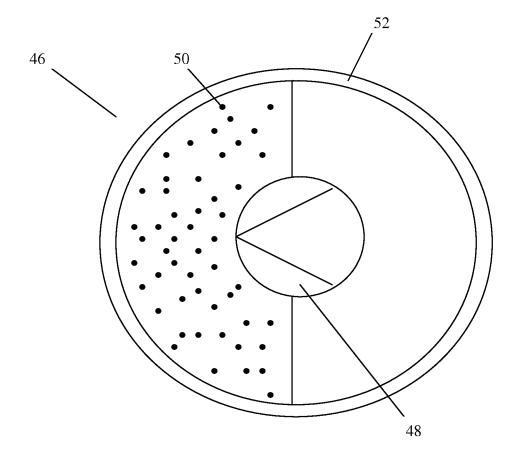


FIG. 5

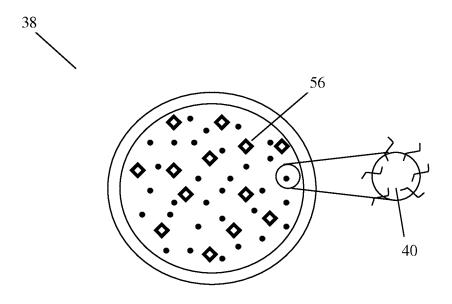


FIG. 6

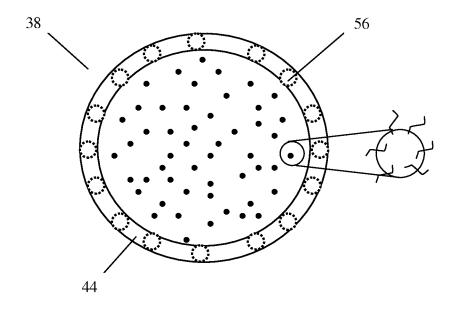


FIG. 7

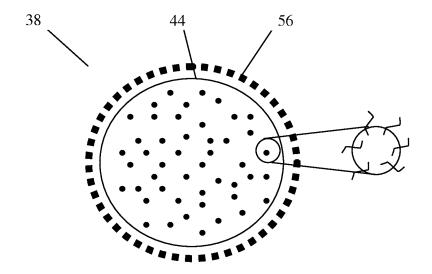


FIG. 8

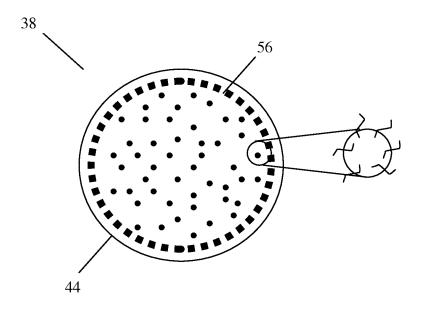


FIG. 9

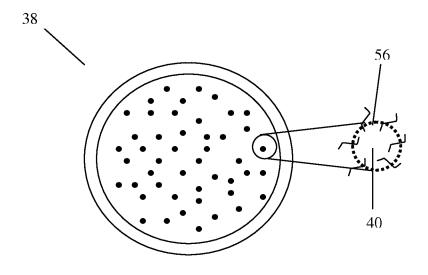


FIG. 10

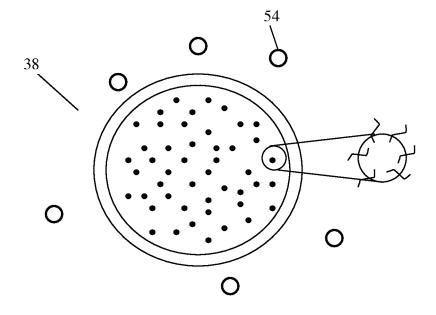


FIG. 11

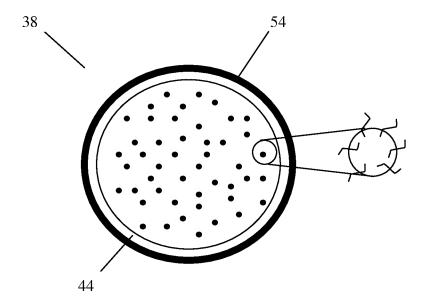


FIG. 12

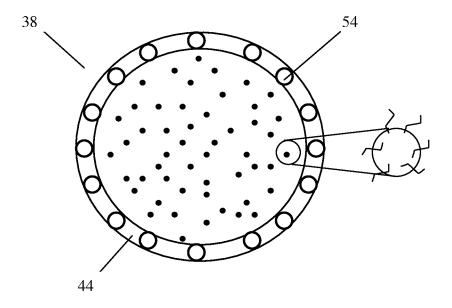


FIG. 13

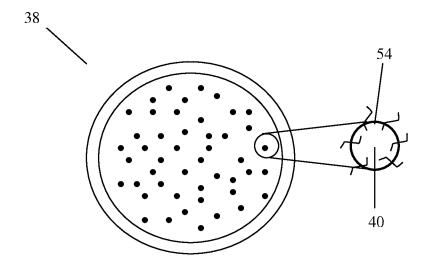


FIG. 14

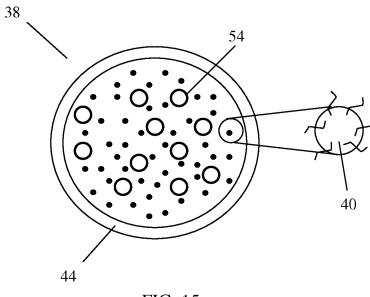


FIG. 15

LOCATION ANALYSIS USING FIRE RETARDANT-PROTECTED NUCLEIC ACID-LABELED TAGS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a divisional of U.S. patent application Ser. No. 12/815,981, filed on Jun. 15, 2010, the entirety of which is hereby incorporated by reference.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to nucleic acid-labeled tags, 15 and, more particularly, to the use of fire retardant to bolster the robustness of nucleic acid-labeled tags.

2. Description of the Related Art

The physical characteristics of a nucleic acid molecule make it uniquely suitable for use as a secure information-storage unit. In addition to being odorless and invisible to the naked eye, a nucleic acid molecule can store vast amounts of information. It has been estimated that a single gram of deoxyribonucleic acid ("DNA") can store as much information as approximately one trillion compact discs ("Computing With 25 DNA" by L. M. Adleman, *Scientific American*, August 1998, pg 34-41).

Nucleic acid molecules are also resilient to decay, even in vitro. Although a nucleic acid molecule typically begins to breakdown when exposed to chemicals, radiation, or ³⁰ enzymes, some nucleic acid molecules can survive for thousands of years. For example, scientists have sequenced the Neanderthal genome using DNA molecules that were recovered from remains dating at least 38,000 years old.

Lastly, nucleic acid molecules are both ubiquitous in nature 35 and largely uncharacterized, with only a fraction of the world's organisms having been sequenced. As a result of this uncharacterized environmental background noise, inadvertent detection of a man-made nucleic acid molecule is unlikely.

To employ the many beneficial characteristics of nucleic acids, these molecules can be incorporated into a secure tag. These tags can be composed of deoxyribonucleotides, ribonucleotides, or similar molecules composed of nucleic acids that are either artificial (such as nucleotide analogues) or are 45 otherwise found in nature. The nucleic acids can range from very short oligonucleotides to complete genomes.

Once a nucleic acid tag is created it can be used for numerous unique security applications including to: (i) detect illicit tampering with physical objects; (ii) secure the privacy of a 50 room or building; (iii) send encoded messages between individuals; (iv) detect a tagged individual or object at a distance; (v) track the recent travel history of an individual or object; or (vi) monitor a location of interest.

DNA tags have previously been used for other applications. For example, DNA tags have been removably attached to tangible assets to assist in the identification of ownership in the event the asset is lost or stolen. Additionally, it has been proposed that DNA tags be used to prevent counterfeiting by incorporating tags into items during or after production and using detection of such tags to authenticate the items.

SUMMARY OF THE INVENTION

It is therefore a principal object and advantage of the 65 present invention to provide a nucleic acid tag that can be used in numerous security-related applications.

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It is a further object and advantage of the present invention to provide a method of standoff detection using nucleic acid tags.

It is yet another object and advantage of the present invention to provide a method of determining whether an object has traveled through a location using seeded nucleic acid-labeled tags.

It is a further object and advantage of the present invention to backtrack or identify an object's point of origin or recent geographic course using seeded nucleic acid-labeled tags.

It is yet another object and advantage of the present invention to provide a fire retardant-protected nucleic acid-labeled tag.

Other objects and advantages of the present invention will in part be obvious, and in part appear hereinafter.

In accordance with the foregoing objects and advantages, the present invention provides for a nucleic acid tag, the tag comprising: (a) a nucleotide-support platform attached to a nucleic acid molecule; (b) a fire retardant; and (c) an encapsulant

Another embodiment of the present invention provides for a nucleic acid tag, the tag comprising: (a) a nucleotide-support platform attached to a nucleic acid molecule; (b) a fire retardant; and (c) an encapsulant, wherein the encapsulant is adapted to prevent degradation of the nucleic acid molecule.

A further embodiment of the present invention provides a method of determine whether an item has moved through a geographic location, the method comprising: (a) creating a nucleic acid tag comprised of a nucleotide-support platform attached to a nucleic acid molecule, and further comprising a fire retardant; (b) seeding the geographic location with the nucleic acid tag; and (c) examining the item for the presence of the nucleic acid tag.

Yet another embodiment of the present invention is a method for backtracking the travel history of an item, the method comprising: (a) creating two or more nucleic acid tags, said nucleic acid tags comprised of a nucleotide-support platform attached to at least one nucleic acid molecule, and further comprising a fire retardant; (b) seeding each of two or more geographic locations with said nucleic acid tags, wherein each geographic location is seeded with a unique nucleic acid tag; (c) examining said item for the presence of one or more nucleic acid tags; and (d) identifying the geographic location associated with each nucleic acid tag detected on the item.

Another embodiment of the present invention is a method for determining the point of origin of an item, the method comprising: (a) creating two or more nucleic acid tags, said nucleic acid tags comprised of a nucleotide-support platform attached to at least one nucleic acid molecule, and further comprising a fire retardant; (b) seeding each of two or more geographic locations with said nucleic acid tags, wherein each geographic location is seeded with a unique nucleic acid tag; (c) examining said item for the presence of one or more nucleic acid tags; and (d) identifying the geographic location associated with each nucleic acid tag detected on the item; and (e) extrapolating the point of origin.

A further embodiment of the present invention provides a method for determining whether an incendiary device has moved through a geographic location, the method comprising: (a) creating a nucleic acid tag comprised of a nucleotide-support platform attached to a nucleic acid molecule and further comprising a fire retardant; (b) seeding the geographic location with the nucleic acid tag; and (c) examining the incendiary device or a fragment thereof for the presence of the nucleic acid tag.

Yet another embodiment of the present invention provides a method for backtracking the travel history of an incendiary device, the method comprising: (a) creating two or more nucleic acid tags, said nucleic acid tags comprised of a nucleotide-support platform attached to at least one nucleic acid molecule and further comprising a fire retardant; (b) seeding each of two or more geographic locations with said nucleic acid tags, wherein each geographic location is seeded with a unique nucleic acid; (c) examining said incendiary device or a fragment thereof for the presence of one or more nucleic acid tags; and (d) identifying the geographic location associated with each nucleic acid tag detected on said incendiary device

Another embodiment of the present invention provides a method for backtracking the travel history of an incendiary 15 device, the method comprising: (a) creating two or more nucleic acid tags, said nucleic acid tags comprised of a nucleotide-support platform attached to at least one nucleic acid molecule and further comprising a fire retardant; (b) seeding each of two or more geographic locations with said nucleic acid tags, wherein each geographic location is seeded with a unique nucleic acid; (c) examining said incendiary device or a fragment thereof for the presence of one or more nucleic acid tags; (d) identifying the geographic location associated with each nucleic acid tag detected on said incendiary device; 25 and (e) extrapolating the point of origin.

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention will be more fully understood and 30 appreciated by reading the following Detailed Description of the Invention in conjunction with the accompanying drawings, in which:

- FIG. 1 is a schematic representation of nucleic acid tag production.
- FIG. 2 is a schematic representation of an embodiment of the method according to the present invention.
- FIG. 3 is a side view of an encapsulated nucleotide tag complex.
- FIG. 4 is a side view of encapsulated nucleotide-deriva- 40 tized nanoparticles.
- FIG. 5 is a side view of an encapsulated tag complex containing a retroreflector and nucleotide-derivatized nanoparticles.
- FIG. 6 is a side view of an encapsulated nucleotide tag 45 complex with fire retardant trapped inside the tag by the encapsulant layer.
- FIG. 7 is a side view of an encapsulated nucleotide tag complex with fire retardant incorporated into the encapsulant layer.
- FIG. **8** is a side view of an encapsulated nucleotide tag complex with fire retardant coating the outer surface of the encapsulant.
- FIG. 9 is a side view of an encapsulated nucleotide tag complex with fire retardant coating the inner surface of the 55 encapsulant.
- FIG. 10 is a side view of an encapsulated nucleotide tag complex with fire retardant incorporated into the nanoparticles.
- FIG. 11 is a side view of an encapsulated nucleotide tag 60 complex with separate marker elements.
- FIG. 12 is a side view of an encapsulated nucleotide tag complex with marker elements coating the outer surface of the encapsulant.
- FIG. 13 is a side view of an encapsulated nucleotide tag 65 complex with marker elements incorporated into the encapsulant layer.

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FIG. 14 is a side view of an encapsulated nucleotide tag complex with marker elements incorporated into the nanoparticles.

FIG. 15 is a side view of an encapsulated nucleotide tag complex with marker elements trapped inside the tag by the encapsulant layer.

DETAILED DESCRIPTION OF THE INVENTION

Referring now to the drawings wherein like reference numerals designate identical or corresponding parts throughout the several views, there is shown in FIG. 1 a schematic representation of nucleic acid tag production. As an initial step 10, a nanometer-sized particle ("nanoparticle") platform is prepared for attachment to the nucleic acid molecule(s). A platform is used to make the nucleic acid more accessible to downstream analysis and prevent nucleic acid loss if any portion of the encapsulating layer is compromised.

The platform is any compound that can be attached to nucleic acid without unintentionally degrading or altering the nucleic acid sequence. For example, the platform can be a lightweight, durable, non-water soluble, and chemically inert structure composed of silica or polystyrene. Additionally, the nanoparticle platform should be composed of a compound that does not inhibit any downstream analysis of the nucleic acid molecules, including tag detection and polymerase chain reaction ("PCR").

In step 12, the nucleic acid molecule is attached to the prepared nanoparticle platform. The nucleic acid molecules are optimally attached to the nanoparticle to facilitate later analysis. In a preferred embodiment, a chemical linker is used to connect the nucleic acid to the nanoparticle platform. This chemical linker must keep the nucleic acid securely tethered to the nanoparticle while avoiding inhibition of the detection or analysis of the tag and nucleic acid. Although the chemical linker can be chosen to provide a permanent covalent link between the nucleic acid and the nanoparticle platform, it could also be a compound that quickly and efficiently releases the nucleic acid at a certain temperature or after exposure to a release compound.

The nucleic acid molecule can also be designed to promote analysis. For example, to avoid steric hindrance or unwanted intermolecular interactions, the molecule can include nucleotide spacers between the chemical linker or nanoparticle base and the information-coding segment of the nucleotide sequence. Spacing between 5 and 15 bases has been optimal for current applications, although this may vary as new applications are considered.

The concentration of nucleic acid molecules on the nanoparticle platform is also an important factor in downstream analysis. If the molecules are too concentrated, steric hindrance prevents the primer and polymerase from efficiently binding the proper segments of the nucleic acid molecules. If the molecules are too sparse, the PCR signal will be diminished and can result in false negatives. In the preferred embodiment, a concentration of about 3×10^{10} nucleic acid molecules per square centimeter is the optimal concentration for robust PCR signal.

In step 14, the nucleic acid-derivatized nanoparticles are agglomerated. Agglomeration protects the nucleic acid molecules from degradation and facilitates encapsulation. To agglomerate the particles to the desired size range, the nanoparticles are vacuum dried, milled, and sieved.

Compounds might be used or incorporated into the tag to promote disagglomeration of the agglomerates prior to PCR analysis. These compounds might be bovine serum albumin, salmon sperm DNA, carbohydrates, polyvinyl alcohol, fruc-

tose, or chitosan, among others. With more nucleic acid exposed during dissolution, subsequent analysis will be faster and more sensitive.

After the nanoparticles are agglomerated, the agglomerates are encapsulated in step 16. The encapsulant protects the 5 nucleic acid from degradation by ultraviolet light, hydrolysis, enzymatic digestions, chemical degradation, or any other means. Additionally, the encapsulant can be designed such that it does not hinder analysis of the nucleic acid molecules. For example, the encapsulant should not contain any compounds that would inhibit or prevent a PCR reaction, although efficient removal of the encapsulant before PCR analysis would eliminate this requirement. Additionally, the encapsulant should enhance the ability of the tag to discretely attach to people and objects. If covertness is required, the encapsulant can be designed to deter detection.

The encapsulating layer can also be designed with surface moieties added to the inner or outer surfaces of the encapsulant or incorporated into the encapsulant material. The moieties are designed to facilitate a particular use of the nucleic 20 acid tag. For example, the moiety can be hydrophobic to enable stickiness or contain antibodies designed for specific targeting. The molecular interactions between the moiety and a target compound can range from simple electrostatic interactions to antibody-antigen recognition. The moiety can also 25 promote detection of the nucleic acid tag.

To protect the nucleic acid from degradation, the encapsulating layer can be coated with or include another functional layer of material. For example, the encapsulant can be coated with or include a non-water-soluble compound to prevent 30 access to water or similar molecules. The encapsulant can also be coated with or include a UV-blocking compound such as titanium dioxide to prevent UV-induced degradation of the nucleic acid molecules.

The tag can also include a flame retardant or fire retardant 35 to improve robustness, as shown in step 17 of FIG. 1. The flame or fire retardant is preferably anything known by those skilled in the art to inhibit combustion or reduce the temperature of associated material in response to high temperatures, including but not limited to Nomex®, GORE-TEX®, Kev- 40 lar®, aluminum hydroxide, magnesium hydroxide, hydromagnesite, calcium silicate, or halocarbons, among many others. While some compounds provide the tag with resistance to combustion, others provide the tag with thermal protection by absorbing heat in an endothermic reaction, 45 through chemical degradation, or by otherwise protecting the tag from high temperatures. The flame retardant can also be modified with other compounds to provide additional desired characteristics including but not limited to color, luminescence, or protection against ultraviolet radiation.

FIG. 2 is a schematic representation of an embodiment of a security method according to the present invention. More specifically, the figure represents characterization of the recent travel history of point of an item. An item can be any person or object of interest. Seeding an area with tags that 55 naturally or artificially adhere to objects (including people or animals) provides a mechanism for identifying the origin of those objects simply by analyzing the adhering tags. Similarly, by seeding different areas with discernibly different tags it is possible to backtrack the geographic path that an object 60 has followed. Such a mechanism would allow the seeder—the person or organization who seeded and will analyze the tags—to identify the recent travel history of the person or object; to quickly identify people or objects that have traveled through seeded areas; and to identify vehicles that have traveled through seeded areas and might carry dangerous cargo such as explosives, among other uses.

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As an initial step 18, an identifiable nucleic acid is characterized or created. In one embodiment of the present invention, the sequence ranges from a short oligonucleotide to an entire genome and is generated through any of the various known methods of natural or artificial nucleic acid synthesis. The nucleic acid can be completely composed of either natural nucleic acids which normally compose the genomes of organisms, artificial nucleic acids, or a mixture of the two.

In the preferred embodiment of the tag, the nucleic acid molecules of each type of tag—which typically differ depending on location or manner of use—contain identical primer-binding sequences surrounding unique nucleotide sequences. Each unique nucleotide sequence contained between the primers encodes information that corresponds to the location, time, or other data specific to that unique sequence. Since analysis of a detected tag uses the same primers, the analysis is performed faster and more efficiently.

The primer sequences, whether they are unique or identical for each location or use, are chosen to avoid cross-reactions with naturally-occurring nucleic acid molecules in the environment in which the tag is located. Although only a fraction of natural nucleic acid molecules on Earth have been characterized by scientists, the search of nucleic acid repository databases such as GenBank®, the National Institutes of Health database containing all publicly available DNA sequences, should be a preliminary step in constructing the primer sequences.

In one embodiment of the current invention, unique groupings of nucleotides are assigned a specific letter, number, or symbol value in order to encode information within the sequence. By placing the unique groupings in order, information can be encrypted into the nucleotide sequence. To further increase the security of the information, advanced encryption algorithms can be used to assign letter, number, or symbol values to specific nucleotides or nucleotide groupings. Additionally, the encryption system can be periodically changed to prevent decryption by intercepting entities.

The nucleic acid can also be encoded to contain information other than a string of letters, numbers, and symbols. For instance, the sequence can be a random sequence that corresponds to the latitude and longitude of the site that will be seeded. Alternatively, the tag can be as simple as a single nucleic acid change in a previously identified or known sequence. For example, the nucleotide sequence can be embedded in a full or partial genomic sequence corresponding to an organism which naturally exists in the location to be seeded. Modifications to the natural nucleic acid sequence, known only to the creator of the tag, can be made such that the changes resemble natural variations of the sequence and thus fail to arouse suspicion, even by individuals that might suspect such tags are present.

To decrypt the encoded information according to this system, an individual will need: (1) knowledge that encoded nucleic acid is present; (2) knowledge of the specific location of the information within the nucleic acid in order to use the appropriate primers for amplification and sequencing reactions; (3) access to a PCR machine and reagents; and (4) the encryption algorithm, or, alternatively, complex decryption capabilities.

Although creating the tag within the genome of an naturally-occurring organism provides numerous benefits, both in vivo and in vitro DNA replication occasionally introduces random errors into a DNA sequence despite the actions of proof-reading and repair enzymes. By deleting one or more nucleotides or frame-shifting the nucleic acid sequence, these mutations can disrupt any encrypted information contained therein. Computer algorithms are used to restore the informa-

tion by recognizing and repairing the errors. For example, if a mutation adds one or more nucleotides to a pre-defined sequence and disrupts the information, the algorithm removes single or multiple nucleotides from the sequence until the information is corrected. Similarly, if a mutation removes one or more nucleotides, the algorithm systematically adds nucleotides to the sequence until the information is corrected. The algorithm must also be robust enough to decrypt sequences that contain more than one type of error-inducing mutation, and must be capable of recognizing when the information contained with the nucleic acid has been restored.

In step 20 of FIG. 1, the nucleic acid is packaged into an appropriate tag complex. To avoid potentially harmful environmental side-effects, the tag can be large enough to avoid being inhaled by people or organisms but small enough to be covert. FIG. 3 represents one embodiment of this tag structure. Tag 30 is composed of a single nucleotide-support platform 32, nucleic acid 34, and encapsulant 36.

FIG. 4 is a side view of another embodiment of the tag 20 structure. Tag 38 is composed of nucleotide-support platform 40 derivatized with nucleic acid 42 and surrounded by encapsulant 44. Similar to the tag in FIG. 3, tag 38 contains nucleic acids that are contained within an encapsulant that protects bead platform used by the tag in FIG. 3, nucleotide-support platform 40 is composed of nanoparticles. Tag 38 can contain thousands, millions, or even billions of nucleotide-derivatized nanoparticles within the encapsulant layer.

FIG. 5 is yet another embodiment of the tag complex. 30 Encapsulated tag 46 contains a retroreflector 48, nucleotidederivatized nanoparticles 50, and encapsulant 52. Retroreflector 48, a device that reflects an electromagnetic wave front back along a vector that is parallel to but opposite in direction from the angle of incidence, forms the center of tag 46. The 35 retroreflector must be situated to allow electromagnetic waves to hit and reflect from the surface. To prevent obstruction of the retroreflector, the tag is organized to keep nucleotide-derivatized nanoparticles 50 away from the surface of the retroreflector, as shown in FIG. 5. Additionally, encapsu- 40 lant 52 must protect the tag complex without interfering with the retroreflector's reflectivity. As an alternative to the nanoparticle format shown in FIG. 5, the nucleic acid can coat the non-reflective surfaces of retroreflector 48. In another embodiment of the retroreflector tag, the non-reflective sur- 45 faces of the retroreflector are coated with nucleic acid and only those surfaces are covered by a protective encapsulant.

In yet another embodiment, the tag complex can include a fire or flame retardant 56 that protects the tag by resisting combustion or lowering high external temperatures. A fire- or 50 high temperature-resistant tag can be used for many different applications, including those where the tag is expected to be exposed to fire or the high temperature of an explosion. The tags can be used to seed areas, components, or individuals suspected to be involved in the constructions of bombs or 55 other incendiary devices, and the fire- or heat-resistant element would help the seeded tag survive the explosion, which could then be analyzed using downstream processes.

The fire retardant element 56 can be incorporated into the tag in a number of different ways. For example, in FIG. 6 the 60 retardant is separate from nucleotide-support platform 40 and encapsulant 44 but is trapped within the interior of tag 38. In FIG. 7, fire retardant **56** is incorporated into encapsulant **44**. In FIG. 8, the retardant forms a layer on the exterior surface of the encapsulant. Retardant 56 could also coat the interior surface of the encapsulant, as shown in FIG. 9. In FIG. 10, the retardant coats the surface of nucleotide-support platform 40.

In several of the described embodiments, the encapsulant layer must be designed to prevent inhibition of excitation and emission wavelengths.

In step 22 of FIG. 2, one or more geographic locations are seeded with the tags. The locations are seeded with tags using any mechanism that will adequately disperse the tags at the desired concentration. For example, the tags can be seeded on and along roadways or paths using an automobile that has been modified to disperse the tags. The tags can also be discretely dispersed from the air using an airplane or remotely-controlled flying apparatus. Tags can even be seeded by individuals using hand-held dispersal systems.

To efficiently backtrack the movements of a person, vehicle, or object, each road within a given location can be seeded with a unique tag. As the vehicle moves through the location it picks up tags from each road it traverses. This system can be scaled up or scaled down to suit the needs of the seeder. For example, rather than seeding individual roads the seeder can use the tags to label large regions of land to backtrack large-scale movements. Alternatively, the seeder can scale down the method by seeding individual homes or buildings to identify individuals or objects that have entered those buildings.

In step 24 of FIG. 2, an item is examined for the presence the sequence without inhibiting later analysis. Unlike the 25 of seeded tags. Once an object of interest is identified, the object can be examined for seeded tags using any mechanism designed to pick up tags from the surfaces of the object. For example, the tires, wheel wells, or underside of a vehicle can be swabbed for tags. If the object of interest is a person, the individual's clothes, shoes, hair, or skin can be swabbed for tags. If the object of interest is a post-blast fragment of an explosive device, the surfaces of the fragment can be swabbed for any tags that survived the explosion.

> If the seeded tags contain retroreflectors, electromagnetic waves can be used to detect the presence of tags. Scanning equipment shines light on the object of interest and looks for a wave front that is reflected along a vector that is parallel to but opposite in direction from the wave's source. This suggests that retroreflective tags are present on the surface of the object and alerts the authorities that further investigation is necessary. This rapid and cost-effective identification of retroreflective tags is especially useful for high-throughput locations such as checkpoints and border crossings. Once the retroreflective tags are detected, they can be removed from the surfaces of the object for analysis of the attached nucleic acids to identify geographic locations.

> The tags can also contain luminescent compounds that reveal their presence from a distance. Although the preferred embodiment uses fluorescent or phosphorescent photoluminescence, other embodiments may include chemiluminescent, radioluminescent, or thermoluminescent compounds. The photoluminescent compound is chosen such that absorption of a photon with a certain wavelength by the compound causes the emission of a photon with a different wavelength. The difference between the wavelength of the absorbed photon and the wavelength of the emitted photon depends on the inherent physical properties of the chosen compound.

> In the preferred embodiment, the luminescent compound absorbs and emits photons in the ultraviolet band—between 400 and 10 nanometers—of the electromagnetic spectrum. The compound is chosen to avoid interference by UV radiation from the sun. The Earth's atmosphere absorbs as much as 99% of the UV radiation emitted by the sun in the 150-320 nm range. Thus the most advantageous luminescent compound absorbs and emits photons with wavelengths below 320 nm.

> As an alternative to luminescent compounds that absorb and emit photons in the 150-320 nm range, compounds that

absorb and emit photons of wavelengths greater than 320 nm can be used under certain circumstances. For example, these compounds could be used during nighttime conditions or in an enclosed UV-blocking environment such as a windowless structure.

The luminescent compound can be incorporated into the tag in a number of different ways. For example, in FIG. 11 the compound 54 is entirely separate from tag 38. In FIG. 12, compound 54 forms a layer on the exterior surface of encapsulant 44. The compound could also coat the interior surface 10 of encapsulant 44. In FIG. 13, compound 54 is incorporated into encapsulant 44. In FIG. 14, compound 54 coats the surface of nucleotide-support platform 40. In FIG. 15, compound 54 is separate from nucleotide-support platform 40 and encapsulant 44 but is trapped within the interior of tag 38. In 15 several of the described embodiments, the encapsulant layer must be designed to prevent inhibition of excitation and emission wavelengths.

If the seeded tags contain a photoluminescent compound, electromagnetic waves can be used to detect the presence of 20 tags at a distance. Scanning equipment shines photons of the excitatory wavelength on the object of interest and looks for photons emitted at the proper wavelength as determined by the compound used in the tags. Detection of photons with the correct wavelength suggests that a nucleic acid-labeled tag is 25 present and alerts the scanner that further investigation is necessary. The advantage of this system is that the scanning equipment and tag can be designed such that the individual doing the scanning does not have to be in close proximity to the object of interest.

The detection process can also be automated. An individual or object of interest can be forced to travel through a scanning point containing excitation equipment and emission detection equipment. As the individual or object of interest travels through the scanning point, the equipment scans for emitted 35 photons of a certain wavelength. When the emitted photons are detected, a computer at the scanning point automatically alerts a remotely-located entity that subsequent analysis is

In yet another embodiment of the current invention, the 40 nucleic acids contained within the tags taken from the surface of an object are analyzed using any method that determines the exact order of nucleotide bases. There are currently a number of different commonly-used sequencing techniques including but not limited to dye-terminator sequencing, par- 45 allel sequencing, and sequencing by ligation. Sequencing machines allow automated sequencing and can be run 24 hours a day. If PCR techniques are used, the appropriate primers are chosen based upon the types of tags known to be in the location of interest. Prior to sequencing or amplifica- 50 through a geographic location, the method comprising: tion, it is necessary to dissolve or otherwise remove the encapsulant layer from the tag in a manner that avoids inhibition of the downstream sequencing or PCR reactions. In the preferred embodiment, the encapsulant and/or agglomerate is disrupted by bead beater, a form of mechanical disruption. 55 This one-step method avoids chemicals or extractions which could affect or inhibit PCR reactions.

In addition to the traditional sequencing techniques described above, real-time PCR and sequencing by hybridization techniques allow rapid detection of target nucleic 60 acids. According to the real-time PCR technique, the extracted nucleic acid is placed into a well or tube that has been pre-loaded with all reagents necessary for a PCR reaction as well as a sequence-specific, nucleotide-based, fluorescently-labeled probe. As the extracted nucleic acid is 65 amplified, the polymerase degrades the probe and releases the fluorescent reporter. The reporter immediately fluoresces and

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alerts the system to the presence of a tag nucleotide. Under the sequencing by hybridization technique, the extracted nucleic acid is labeled with a fluorescent marker and is hybridized to a DNA microarray that contains the complementary nucleotide sequence from known seeded tags. If the extracted nucleic acid hybridizes to any of the complementary tags, the fluorescent signal alerts the system to the presence of a target nucleic acid. Since both methods of analysis avoid additional analysis and require relatively inexpensive analytical equipment, they promote faster and more affordable generation of data and require

In step 26 of FIG. 2, the sequences obtained from the identified tags are compared to a database of sequences attached to seeded tags. To efficiently determine the point of origin or recent travel history of an object, the individuals analyzing tags detected in the field will need access or information about the tags dispersed by the seeder. A database of seeded tags will require maximum security measures to avoid improper access and manipulation, including access protection measures such as passwords. Standard computer algorithms are used to find exact or approximate matches between a sequence in the field and a tag sequence in the database. Once such a match is found, the user can reasonably suspect that the object of interest has recently traveled through the location seeded by that tag. If the real-time PCR or sequencing by hybridization techniques are used, the identification of the seeded tags is quickly determined by equipment that scans the plate or microarray for fluorescent label.

Step 28 of FIG. 2 is an optional step which is only required if the user is attempting to backtrack the route taken by an object of interest or extrapolate the object's point of origin. According to some uses of the present invention, simply learning that a person or object has traveled through a particular location is sufficient information. For other uses, it is necessary to analyze the sequences of multiple tags. To extrapolate a route taken or a point of origin, the seeded tag location information obtained by analyzing the surfaces of the object is fed into a computer algorithm that quickly plots every potential route that the object has traveled based upon the possible combinations of tag locations. A similar algorithm can be used to extrapolate a point of origin based upon the identified tag locations.

Although the present invention has been described in connection with a preferred embodiment, it should be understood that modifications, alterations, and additions can be made to the invention without departing from the scope of the invention as defined by the claims.

What is claimed is:

analogues.

1. A method for determining whether an item has moved

creating a nucleic acid tag comprising: an agglomerated plurality of nanoparticle nucleotide-support platforms each attached to a plurality of nucleic acid molecules, each of said nucleic acid molecules comprising identifying information, wherein a spacer is located between said nanoparticle nucleotide-support platform and said identifying information; a fire retardant; and an encapsulant surrounding said agglomerated plurality of nanoparticle nucleotide-support platforms and said plurality of nucleic acid molecules;

seeding the geographic location with the nucleic acid tag;

examining the item for the presence of the nucleic acid tag. 2. The method of claim 1, wherein each nucleic acid molecule comprises nucleotides selected from the group consisting of ribonucleotides, deoxyribonucleotides, and nucleotide

3. The method of claim 1, wherein said identifying information is encrypted within the nucleic acid molecules by altering the sequence of nucleotides.

- 4. The method of claim 1, wherein at least some of said nucleic acid tags comprise a retroreflector.
- ${\bf 5}.$ The method of claim 1, wherein the item is an incendiary device.
- 6. The method of claim 1, wherein the item is a fragment of an incendiary device.
- 7. The method of claim 1, wherein the nucleic acid tag is 10 analyzed by sequencing all or part of the nucleic acid molecule.
- **8**. The method of claim **1**, wherein each geographic location is seeded with a unique nucleic acid tag.

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